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**Article:**

Rothman, A.M., Rowlands, D.J. and Lawrie, A. [orcid.org/0000-0003-4192-9505](https://orcid.org/0000-0003-4192-9505) (2016)  
*miRNA-140-5p: new avenue for pulmonary arterial hypertension drug development?*  
Epigenomics. ISSN 1750-1911

<https://doi.org/10.2217/epi-2016-0089>

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## **MicroRNA-140-5p: new avenue for pulmonary arterial hypertension drug development?**

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**Keywords:** MicroRNA, miR140-5p, SMURF1, Ubiquitination, Pulmonary Arterial Hypertension, Remodelling

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## **Abstract**

Pulmonary arterial hypertension (PAH) is a rare but fatal disease. Pathologically, PAH is characterised by sustained vasoconstriction and progressive obliteration of small pulmonary arteries through a process of medial thickening, intimal fibrosis and the formation of angioproliferative lesions. Current treatments target the sustained vasoconstriction via either the prostacyclin, endothelin or nitric oxide pathway but do little to address the underlying progressive proliferative vascular disease. Dysregulated expression of microRNA (miR) has been identified in PAH and we have recently highlighted reduced miR-140-5p in patients with PAH. Replacement of miR-140-5p attenuated disease in animal models with the regulation of Smurf1, a E3 ubiquitin ligase targeting BMPR2 as one identified mechanism. These data highlight Smurf1 inhibition as a treatment for PAH.

## **Editorial**

Pulmonary arterial hypertension (PAH) is a progressive, fatal disease characterised by occlusive remodelling of the small pulmonary arteries within the lung. The complex pathology of PAH is characterised by sustained pulmonary vasoconstriction, early pulmonary artery endothelial cell (PAEC) dysfunction/leak, proliferation of pulmonary artery smooth muscle (PASMC), emergence of apoptosis resistance PAEC and the recruitment of circulating inflammatory cells [1]. Untreated, the disease carries a median life expectancy of less than 3 years [2]. Current pharmacological treatments are primarily vasodilators, as their mechanism of action and offer a significant increase in survival but there remains no cure other than transplantation.

Since the identification of mutations in the gene encoding bone morphogenetic protein receptor type 2 (BMPR2) in familial cases of PAH over 15 years ago [3], there are now well-established additional mechanistic insights into disease pathogenesis including interaction with growth factors including PDGF, serotonin pathway, inflammation including, IL-6 [4,5], TNF superfamily members osteoprotegerin [6-8] and TRAIL[9,10], mitochondria metabolism, oestrogen as well as epigenetic regulation of these pathways [1,11]. BMPR2 mutations are estimated to be present in up to 80% of patients with heritable PAH and 30% of Idiopathic PAH, and confer a worse prognosis [12] but penetrance of disease in mutation carriers is low. Subsequently, the precise cell and molecular mechanisms leading to disease

manifestation, and driving pathogenesis remain poorly understood. Dissecting the molecular mechanisms underlying PAH is therefore crucial if effective treatments for a condition that has a worse prognosis than many malignancies need to be developed.

The dysregulation of a number of microRNA (miR) has been identified in both patients with PAH, and investigated in animal models of the disease [13]. Several of these miR provide mechanistic insight [14] and prognostic utility [15] but discordance between models and clinical samples have reported [16].

We have recently published our research describing reduced levels of miR-140-5p in patients with PAH, and animal models of PAH [17]. Whole blood expression of miR-140-5p correlated with clinical measures of disease severity, and predicted survival. To determine whether miR-140-5p was important in disease progression, or merely a bystander we performed a series of pre-clinical studies where we delivered synthetic miR-140-5p packaged within liposomes nebulised directly to the lung. We first looked at whether prophylactic treatment concurrent with disease initiation, followed by therapeutic administration in rats where disease was first allowed to manifest utilising two rat models. In all cases rats treated with the miR-140-5p and demonstrated a significant attenuation of disease phenotype compared to those treated with a scrambled miR sequence. The benefit on disease phenotype was observed in both a reduction in haemodynamic indices of PAH and a reduction in the severity of pulmonary vascular remodelling.

Bioinformatic prediction of miR-140-5p targets identified an overrepresentation of TGF beta and BMP signalling related mRNA including several important genes previously associated with PAH including the PDGFalpha receptor and VEGF. The most connected target within this network, and the one with the most conserved miR-140-5p binding site was *Smurf1 mRNA*. Smurf1 is a E3 ubiquitin ligase that targets type I BMP receptors (including BMPR2 [18]) and downstream signalling mediators [19] for ubiquitination and degradation. Through these actions Smurf1 acts a negative regulator of BMPR2 signalling. We therefore sought to determine whether one of the mechanisms by which miR-140-5p regulates PAH pathogenesis was via Smurf1. In lung tissue from miR-140-5p and control animals we demonstrated that there was an inverse correlation between levels of miR-140-5p and Smurf1. Further increasing confidence that Smurf1 is important in human disease examination of explanted lung tissue from patients with idiopathic and heritable PAH demonstrated

increased expression of Smurf1 within the pulmonary vasculature. A miR can regulate target mRNA through direct targeting of the 3' untranslated region or non-direct mechanisms. To demonstrate that the Smurf1 3' untranslated region is regulated by binding miR-140-5p directly we modulated expression a reporter construct with a luciferase gene upstream of the Smurf1 3'UTR by co-transfection with miR-140-5p mimic and inhibitor in human PASMC. We also demonstrated augmentation of BMP signalling with miR-140-5p mimic and SMURF1 inhibition (siRNA) in PASMC by modulation of expression of a BMP response element luciferase construct (ID1 promoter).

These experiments demonstrated that miR-140-5p is reduced in PAH and that it acts directly on Smurf1 to modulate BMP signalling in a disease relevant cell type. To determine the role of Smurf1 on the development of PAH we induced disease in C57BL6 mice. Deletion of Smurf1 provided an allele dependent protection from the development of disease, with appropriate reduction in right ventricular hypertrophy and remodelling of the small vessels of the pulmonary arterial system.

The first therapy targeting the ubiquitin proteasome pathway, Bortezomib a proteasome inhibitor, was approved by the FDA in 2003 yet no further therapies targeting other aspects of the ubiquitin proteasome pathway have emerged. Despite the promise afforded by Bortezomib, in part due to complex structural diversity, complex mechanisms of regulation and unknowns in translating biochemical activity to functional cellular effect on disease-relevant mechanisms, significant barriers in targeting other components of the proteasome pathway, notably E3 ligases still remain. The HECT family members of E3 ligases such as Smurf1 typically demonstrate a higher degree of catalytic activity than other E3 ligase families suggesting the potential to exploit conventional enzyme inhibitor mechanisms to block substrate ubiquitination [20]. In the context of therapeutic value for PAH patients, an inhibitor for Smurf1 is highly attractive whereby ubiquitination of key substrates within the BMP pathway such as BMPR2 may be inhibited restoring signalling through this key pathway [17]. Compared to a therapeutic miR140-5p mimetic, an inhibitor of Smurf1 may be more tractable where challenges such as rapid miR degradation, low cellular penetration and target engagement particularly in complex remodelled pulmonary vasculature of PAH patients and potential for liver toxicity with miR therapeutics all may limit ability to progress promising molecules to the clinic and ultimately represent barriers to achieving clinical efficacy. Although Smurf1 is reported to have up to 89 different protein substrates [21] suggesting a

complex network of pathways may be modulated by a Smurf1 inhibitor, such pathway interactions are anticipated to be context- and location dependent with Smurf1 protein being enhanced in selected cell types by specific disease stimuli as evident from immunohistochemistry performed on lung tissue from PAH patients. Additionally, the number of potential pathways modulated by a Smurf1 inhibitor are ultimately represent a smaller subset of pathways that targeting miR140-5p which in addition to Smurf1, also regulates expression of PDGFR and ALK5. Together, these data suggest that a selective Smurf1 inhibitor may be a suitable novel therapy for PAH.

**Financial disclosures:** None.

**Acknowledgements:** The authors would like to thank the patients and their families who provides samples to the Sheffield Pulmonary Hypertension Biobank.

## References

1. Schermuly RT, Ghofrani HA, Wilkins MR, Grimminger F. Mechanisms of disease: pulmonary arterial hypertension. *Nature Reviews Cardiology*. 8(8), 443–455 (2011).
2. D'Alonzo G, Barst RJ, Ayres SM, *et al.* Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med*. 115(5), 343–349 (1991).
3. Lane K, Machado R, Pauciulo M, *et al.* Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nature Genetics*. 26, 81–84 (2000).
4. Steiner MK, Syrkina OL, Kolliputi N, Mark EJ, Hales CA, Waxman AB. Interleukin-6 overexpression induces pulmonary hypertension. *Circ Res*. 104(2), 236–44– 28p following 244 (2009).
5. Soon E, Holmes A, Treacy C, *et al.* Elevated Levels of Inflammatory Cytokines Predict Survival in Idiopathic and Familial Pulmonary Arterial Hypertension. *CIRCULATIONAHA*.109.933762 (2010).
6. Lawrie A, Waterman E, Southwood M, *et al.* Evidence of a role for osteoprotegerin in the pathogenesis of pulmonary arterial hypertension. *Am J Pathol*. 172(1), 256–264 (2008).
7. Condliffe R, Pickworth JA, Hopkinson K, *et al.* Serum osteoprotegerin is

- increased and predicts survival in idiopathic pulmonary arterial hypertension. *Pulm Circ.* 2(1), 21–27 (2012).
8. Dawson S, Lawrie A. From bones to blood pressure, developing novel biologic approaches targeting the osteoprotegein pathway for pulmonary vascular disease. *Pharmacol Ther.* 1–5 (2016).
  9. Hameed AG, Arnold ND, Chamberlain J, *et al.* Inhibition of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) reverses experimental pulmonary hypertension. *Journal of Experimental Medicine.* 209(11), 1919–1935 (2012).
  10. Lawrie A. The role of the osteoprotegerin/tumor necrosis factor related apoptosis-inducing ligand axis in the pathogenesis of pulmonary arterial hypertension. *Vascul Pharmacol.* 63(3), 114–117 (2014).
  11. Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest.* 118(7), 2372–2379 (2008).
  12. Sankelo M, Flanagan J, Machado R, *et al.* BMPR2 mutations have short lifetime expectancy in primary pulmonary hypertension. *Hum Mutat.* 26(2), 119–124 (2005).
  13. Rothman AMK, Chico TJA, Lawrie A. MicroRNA in Pulmonary Vascular Disease. In: *Progress in Molecular Biology and Translational Science.* Chico TJA (Ed.). Elsevier, 43–63 (2014).
  14. Bertero T, Lu Y, Annis S, *et al.* Systems-level regulation of microRNA networks by miR-130/301 promotes pulmonary hypertension. *J Clin Invest.* (2014).
  15. Rhodes CJ, Wharton J, Boon RA, *et al.* Reduced MicroRNA-150 Is Associated with Poor Survival in Pulmonary Arterial Hypertension. *American journal of respiratory and critical care medicine.* 187(3), 294–302 (2013).
  16. Schlosser K, Taha M, Deng Y, Jiang B, Stewart DJ. Discordant Regulation of microRNA Between Multiple Experimental Models and Human Pulmonary Hypertension. *Chest.* 148(2), 481–10 (2015).
  17. Rothman AMK, Arnold ND, Pickworth JA, *et al.* MicroRNA-140-5p and SMURF1 regulate pulmonary arterial hypertension. *J Clin Invest.* 126(7) (2016).
  18. Murakami K, Mathew R, Huang J, *et al.* Smurf1 ubiquitin ligase causes downregulation of BMP receptors and is induced in monocrotaline and hypoxia models of pulmonary arterial hypertension. *Exp. Biol. Med. (Maywood).* 235(7), 805–813 (2010).
  19. Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH. A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. - PubMed - NCBI. *Nature.* 400(6745), 687–693 (1999).
  20. Landré V, Rotblat B, Melino S, Bernassola F, Melino G. Screening for E3-Ubiquitin ligase inhibitors: challenges and opportunities. *Oncotarget.* 5(18), 7988–8013 (2014).

21. Andrews PS, Schneider S, Yang E, *et al.* Identification of Substrates of SMURF1 Ubiquitin Ligase Activity Utilizing Protein Microarrays. <http://dx.doi.org/10.1089/adt.2009.0264>. 8(4), 471–487 (2010).